

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	2	"6656726".pn.	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2006/05/15 11:01
L2	43	126/183	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2006/05/15 11:01
L3	32	126/183 and viral	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2006/05/15 11:01
L4	32	126/183 and viral and plant	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2006/05/15 11:01



US005977438A

United States Patent [19]

Turpen et al.

[11] **Patent Number:** 5,977,438[45] **Date of Patent:** Nov. 2, 1999[54] **PRODUCTION OF PEPTIDES IN PLANTS AS VIRAL COAT PROTEIN FUSIONS**[75] **Inventors:** Thomas H. Turpen, Vacaville; Stephen J. Reinl, Sacramento; Laurence K. Grill, Vacaville, all of Calif.[73] **Assignee:** Biosource Technologies, Inc., Vacaville, Calif.[21] **Appl. No.:** 08/324,003[22] **Filed:** Oct. 14, 1994**Related U.S. Application Data**

[63] Continuation-in-part of application No. 08/176,414, Dec. 29, 1993, Pat. No. 5,811,653, and application No. 08/184,237, Jan. 19, 1994, Pat. No. 5,589,367, said application No. 08/176,414, is a continuation-in-part of application No. 07/997,733, Dec. 30, 1992, abandoned, said application No. 08/184,237, is a continuation-in-part of application No. 07/997,733, Dec. 30, 1992, abandoned, which is a continuation of application No. 07/923,692, Jul. 31, 1992, Pat. No. 5,316,931, which is a continuation-in-part of application No. 07/600,244, Oct. 22, 1990, abandoned, application No. 07/641,617, Jan. 16, 1991, abandoned, application No. 07/737,899, Jul. 26, 1991, abandoned, and application No. 07/739,143, Aug. 1, 1991, abandoned, said application No. 07/600,244, is a continuation of application No. 07/310,881, Feb. 17, 1989, abandoned, which is a continuation-in-part of application No. 07/160,766, Feb. 26, 1988, abandoned, and application No. 07/160,771, Feb. 26, 1988, abandoned, said application No. 07/641,617, is a continuation of application No. 07/347,637, May 5, 1989, abandoned, said application No. 07/737,899, is a continuation of application No. 07/363,138, Jun. 8, 1989, abandoned, which is a continuation-in-part of application No. 07/219,279, Jul. 15, 1988, abandoned, said application No. 07/739,143, is a continuation-in-part of application No. 07/600,244, Oct. 22, 1990, abandoned, and application No. 07/641,617, Jan. 16, 1991, abandoned, and application No. 07/737,899, Jul. 26, 1991, abandoned.

[51] **Int. Cl.⁶** A01H 5/00; C12N 5/04; C12N 15/40; C12N 15/62; C12N 15/83[52] **U.S. Cl.** 800/288; 800/278; 800/298; 536/23.4; 536/23.5; 536/23.72; 435/69.7; 435/70.1; 435/235.1; 435/419; 435/468[58] **Field of Search** 536/23.4, 23.5, 536/23.72, 24.1; 435/69.1, 70.1, 172.3, 235.1, 240.4, 418, 419, 69.7, 468; 800/205, 278, 288, 298[56] **References Cited****U.S. PATENT DOCUMENTS**

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(List continued on next page.)

Primary Examiner—David T. Fox**Attorney, Agent, or Firm**—Albert P. Halluin; John A. Bendrick; Howrey & Simon[57] **ABSTRACT**

The present invention relates to foreign peptide sequences fused to recombinant plant viral structural proteins and a method of their production. Fusion proteins are economically synthesized in plants at high levels by biologically contained tobamoviruses. The fusion proteins of the invention have many uses. Such uses include use as antigens for inducing the production of antibodies having desired binding properties, e.g., protective antibodies, or for use as vaccine antigens for the induction of protective immunity, including immunity against parasitic infections.

19 Claims, 10 Drawing Sheets

-continued

Leu	Ile	Val	Glu	Leu	Ile	Arg	Gly	Thr	Gly	Ser	Tyr	Asn	Arg	Ser	Ser
130						135					140				
Phe	Glu	Ser	Ser	Ser	Gly	Leu	Val	Trp	Thr	Ser					
145					150					155					

What is claimed is:

1. A polynucleotide encoding a fusion protein capable of being expressed in a plant or a plant cell, wherein the fusion protein comprises a plant viral coat protein from a single-stranded plus-sense RNA virus fused to a protein of interest comprising four or more amino acids, said polynucleotide further comprising a promoter functional in plants 5' to the fusion protein encoding region.
2. A polynucleotide according to claim 1, encoding a fusion protein wherein the protein of interest is amino-terminal to the plant viral coat protein.
3. A polynucleotide according to claim 1, encoding a fusion protein wherein the protein of interest is carboxy-terminal to the plant viral coat protein.
4. A polynucleotide according to claim 1, wherein said fusion protein is an internal fusion protein.
5. A polynucleotide according to claim 1, further comprising a fusion joint having a leaky stop codon from a single-stranded plus-sense RNA virus.
6. A polynucleotide according to claim 1, wherein the protein of interest is an antigen.
7. A polynucleotide according to claim 1, wherein the coat protein is a tobacco mosaic virus coat protein.
8. A recombinant plant viral genome comprising a polynucleotide according to claim 1.
9. A recombinant plant virus particle, comprising a genome according to claim 8.
10. A recombinant plant virus, wherein the coat protein is encoded by a polynucleotide according to claim 1.
11. A plant cell comprising a polynucleotide according to claim 8.
12. A polynucleotide according to claim 1 wherein the coat protein is a tobamovirus coat protein.
13. A plant cell comprising a recombinant plant viral genome according to claim 8.
14. A plant cell comprising a recombinant plant virus particle according to claim 9.
15. A plant cell comprising a recombinant plant virus according to claim 10.
16. A plant comprising a polynucleotide according to claim 1.
17. A plant comprising a recombinant plant viral genome according to claim 8.
18. A plant comprising a recombinant plant virus particle according to claim 9.
19. A plant comprising a recombinant plant virus according to claim 10.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,977,438
DATED : November 2, 1999
INVENTOR(S) : Thomas H. Turpen, et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 2:

Line 43, change "plan" to --plant--;

Column 7:

Line 15, change "alciparum" to --falciparum--;

Line 18, change "accines" to --vaccines--;

Column 35:

Line 11, change "plane" to --plant--; and

Line 27, change "pluse-sense" to --plus-sense--.

Signed and Sealed this

Nineteenth Day of June, 2001

Attest:

Nicholas P. Godici

Attesting Officer

NICHOLAS P. GODICI
Acting Director of the United States Patent and Trademark Office

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UPDATE

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**PALM INTRANET**

Continuity Information for 10/624193

Parent Data

10624193

is a continuation of 09565616

Which Claims Priority from Provisional Application 60132697

Child Data

No Child Data

[Appln Info](#)[Contents](#)[Petition Info](#)[Atty/Agent Info](#)**Continuity
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**PALM INTRANET**

Inventor Information for 10/624193

Inventor Name	City	State/Country
FITZMAURICE, WAYNE P.	VACAVILLE	CALIFORNIA
POGUE, GREGORY P.	VACAVILLE	CALIFORNIA
LINDBO, JOHN A.	VACAVILLE	CALIFORNIA

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PALM INTRANET

Inventor Name Search Result

Your Search was:

Last Name = LINDBO

First Name = JOHN

Application#	Patent#	Status	Date Filed	Title	Inventor Name
09502711	6300134	150	02/11/2000	RNA transformation vectors derived from a single-component RNA virus and contain an intervening sequence between the cap and the 5'end	LINDBO, JOHN
60316793	Not Issued	159	08/31/2001	Methods for producing human glycoproteins in transfected plants using RNA viral vectors	LINDBO, JOHN
60386891	Not Issued	159	06/07/2002	Method for correlating gene function to sequence location using a sequence lineage evaluation interface	LINDBO, JOHN
09502710	6300133	150	02/11/2000	Rna transformation vectors derived from an uncapped single-componet rna virus	LINDBO, JOHN A.
09520967	6730306	150	03/08/2000	PARVOVIRUS VACCINE AS VIRAL COAT PROTEIN FUSIONS	LINDBO, JOHN A.
09522900	Not Issued	71	03/10/2000	Self antigen vaccines for treating B cell lymphomas and other cancers	LINDBO, JOHN A.
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09565210	Not Issued	161	05/04/2000	Viral expression vectors	LINDBO, JOHN A.
09565616	6656726	150	05/04/2000	VIRAL EXPRESSION VECTORS	LINDBO, JOHN A.
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09775049	Not	161	01/31/2001	Methods for homology-driven	LINDBO, JOHN A.

	Issued			reassembly of nucleic acid sequences	
<u>09823936</u>	Not Issued	161	03/29/2001	Production of peptides in plants as N-terminal viral coat protein fusions	LINDBO, JOHN A.
<u>09949316</u>	Not Issued	161	09/07/2001	RNA transformation vectors derived from a single-component RNA virus and contain an intervening sequence between the cap and the 5' end	LINDBO, JOHN A.
<u>09949317</u>	Not Issued	161	09/07/2001	RNA transformation vectors derived from an uncapped single-component RNA virus	LINDBO, JOHN A.
<u>10057558</u>	Not Issued	161	01/25/2002	Method for enhancing RNA or protein production using non-native 5' untranslated sequences in recombinant viral nucleic acids	LINDBO, JOHN A.
<u>10061216</u>	Not Issued	41	02/04/2002	Production of a parvovirus vaccine in plants as viral coat protein fusions	LINDBO, JOHN A.
<u>10066390</u>	Not Issued	120	02/01/2002	Method of increasing complementarity in a heteroduplex	LINDBO, JOHN A.
<u>10067790</u>	Not Issued	95	02/08/2002	SELF ANTIGEN VACCINES FOR TREATING B CELL LYMPHOMAS AND OTHER CANCERS	LINDBO, JOHN A.
<u>10067892</u>	Not Issued	80	02/08/2002	Self antigen vaccines for treating B cell lymphomas and other cancers	LINDBO, JOHN A.
<u>10067893</u>	Not Issued	161	02/08/2002	Self antigen vaccines for treating B cell lymphomas and other cancers	LINDBO, JOHN A.
<u>10072438</u>	Not Issued	161	02/05/2002	Method of determining the function of nucleotide sequences and the proteins they encode by transfecting the same into a host	LINDBO, JOHN A.
<u>10128510</u>	Not Issued	161	04/24/2002	Production of a parvovirus vaccine in plants as viral coat protein fusions	LINDBO, JOHN A.
<u>10134493</u>	Not Issued	161	04/30/2002	Production of a parvovirus vaccine in plants as viral coat protein fusions	LINDBO, JOHN A.
<u>10190451</u>	Not Issued	61	07/02/2002	Sticky rice	LINDBO, JOHN A.

<u>10193142</u>	Not Issued	93	07/12/2002	PRODUCTION OF A PARVOVIRUS VACCINE IN PLANTS AS VIRAL COAT PROTEIN FUSIONS	LINDBO, JOHN A.
<u>10196677</u>	Not Issued	161	07/15/2002	Method for constructing viral nucleic acids in a cell-free manner	LINDBO, JOHN A.
<u>10200051</u>	Not Issued	161	07/18/2002	Single-component RNA vectors derived from a virus and containing an intervening sequence between the cap and the 5' end and able to replicate in a host plant cell within a host plant	LINDBO, JOHN A.
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<u>10280913</u>	Not Issued	41	10/25/2002	Population of polynucleotide sequence variants	LINDBO, JOHN A.
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<u>10286549</u>	Not Issued	41	11/01/2002	Sticky rice	LINDBO, JOHN A.
<u>10309756</u>	Not Issued	161	12/04/2002	Flexible method and apparatus for high throughput production and purification of multiple proteins	LINDBO, JOHN A.
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<u>10356708</u>	Not Issued	95	01/31/2003	MISMATCH ENDONUCLEASES AND METHODS OF USE	LINDBO, JOHN A.
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<u>10624193</u>	Not Issued	71	07/21/2003	Viral expression vectors	LINDBO, JOHN A.
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<u>10684134</u>	Not	41	10/10/2003	Polynucleotide sequence variants	LINDBO, JOHN A.

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10858775	Not Issued	161	06/01/2004	Method for enhancing RNA or protein production using non-native 5' untranslated sequences in recombinant viral nucleic acids	LINDBO, JOHN A.
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11209592	Not Issued	30	08/22/2005	Self antigen vaccines for treating B-cell lymphomas and other cancers	LINDBO, JOHN A.
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60209893	Not Issued	159	06/06/2000	Construction of a TMV based expression vector	LINDBO, JOHN A.
60266386	Not Issued	159	02/02/2001	Method for reassorting mutations among multiple DNA sequences by in vitro DNA mismatch repair (DMMR-reassortment)	LINDBO, JOHN A.
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60296610	Not Issued	159	06/06/2001	Construction of a TMV based expression vector	LINDBO, JOHN A.

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US006656726B1

(12) **United States Patent**
Fitzmaurice et al.

(10) Patent No.: **US 6,656,726 B1**
(45) Date of Patent: **Dec. 2, 2003**

(54) **VIRAL EXPRESSION VECTORS**

(75) Inventors: **Wayne P. Fitzmaurice**, Vacaville, CA (US); **Gregory P. Pogue**, Vacaville, CA (US); **John A. Lindbo**, Vacaville, CA (US)

(73) Assignee: **Large Scale Biology Corporation**, Vacaville, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/565,616**

(22) Filed: **May 4, 2000**

Related U.S. Application Data

(60) Provisional application No. 60/132,697, filed on May 4, 1999.

(51) Int. Cl.⁷ **C12N 15/00; C12N 5/00; C12N 5/04; C12N 15/82**

(52) U.S. Cl. **435/320.1; 435/410; 435/419; 435/468**

(58) Field of Search **536/23.2, 23.72; 800/295, 278, 298, 317.3; 435/419, 468, 410, 414, 320.1**

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Primary Examiner—Deborah Crouch

Assistant Examiner—Joseph Weitach

(74) *Attorney, Agent, or Firm*—John C. Robbins; Thomas Gallegos; Quine Intellectual Property Law Group, P.C.

(57) **ABSTRACT**

The present invention provides nucleic acid sequences having an altered viral movement protein and 126/183 kDa replicase proteins further characterized in its ability to stabilize a transgene contained in a virus that expresses the altered movement protein. The present invention also provides viral vectors expressing the altered movement protein, cells transformed with the vectors, and host plants infected by the viral vectors.

14 Claims, 17 Drawing Sheets

-continued

Asn Ser Ser Ser Asp Arg Ser Val Pro Asn Lys Asn Tyr Arg Asn Val
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 245 250 255
 Asp Ser Glu Ala Thr Val Ala Glu Ser Asp Ser Phe
 260 265
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 <212> TYPE: PRT
 <213> ORGANISM: Nicotiana tabacum
 <400> SEQUENCE: 6
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 Ser Val Met Cys Ser Lys Val Asp Lys Ile Met Val His Glu Asn Glu
 35 40 45
 Ser Leu Ser Gly Val Asn Leu Leu Lys Gly Val Lys Leu Ile Asp Ser
 50 55 60
 Gly Tyr Val Cys Leu Ala Gly Leu Val Val Thr Gly Glu Trp Asn Leu
 65 70 75 80
 Pro Asp Asn Cys Arg Gly Gly Val Ser Val Cys Leu Val Asp Lys Arg
 85 90 95
 Met Glu Arg Ala Asp Glu Ala Ile Leu Gly Ser Tyr Tyr Thr Ala Ala
 100 105 110
 Ala Lys Lys Arg Phe Gln Phe Lys Val Val Pro Asn Tyr Ala Ile Thr
 115 120 125
 Thr Gln Asp Ala Met Arg Asn Val Trp Gln Val Leu Val Asn Ile Arg
 130 135 140
 Asn Val Lys Met Ser Ala Gly Phe Cys Pro Leu Ser Leu Glu Phe Val
 145 150 155 160
 Ser Val Cys Ile Val Tyr Arg Asn Asn Ile Lys Leu Gly Leu Arg Glu
 165 170 175
 Lys Ile Thr Asn Val Arg Asp Gly Gly Pro Met Glu Leu Thr Glu Glu
 180 185 190
 Val Val Asp Glu Phe Met Glu Asp Val Pro Met Ser Ile Arg Leu Ala
 195 200 205
 Lys Phe Arg Ser Arg Thr Gly Lys Lys Ser Asp Val Arg Lys Gly Lys
 210 215 220
 Asn Ser Ser Ser Asp Arg Ser Val Pro Asn Lys Asn Tyr Arg Asn Val
 225 230 235 240
 Lys Asp Phe Gly Gly Met Ser Phe Lys Lys Asn Asn Leu Ile Asp Asp
 245 250 255
 Asp Ser Glu Ala Thr Val Ala Glu Ser Asp Ser Phe
 260 265

What is claimed is:

1. An isolated nucleic acid sequence comprising a nucleic acid sequence encoding an altered viral movement protein having the amino acid sequence shown in SEQ ID NO: 6.
2. The isolated nucleic acid sequence of claim 1 that is identical to the sequence shown in SEQ ID NO: 4.
3. The isolated nucleic acid of claim 1, wherein the altered movement protein enhances the ability to facilitate stabili-

60 zation of a transgene contained in a virus that expresses the altered movement protein.

4. An isolated nucleic acid sequence comprising a nucleic acid sequence encoding an altered 126/183 replicase complex having a nucleic acid alteration at nucleotide positions 1138, 1268, 2382, and 3632 as shown in SEQ ID NO: 2.

5. An isolated nucleic acid according to claim 4 wherein the altered 126/183 replicase complex enhances the stabili-

31

zation of a transgene contained in a virus that expresses the altered replicase complex.

6. A viral vector comprising a nucleic acid sequence encoding an altered viral movement protein having the amino acid sequence shown in SEQ ID NO.: 6.

7. The viral vector of claim 6, further comprising a transgene, wherein said viral vector exhibits an enhanced ability to stabilize said transgene compared to a control viral vector comprising a wild type movement protein as shown in SEQ ID NO: 3.

8. The viral vector of claim 7, wherein the transgene is a non-viral gene.

9. The viral vector of claim 8, wherein the non-viral transgene encodes a protein selected from the group con-

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sisting of a membrane protein, a cytosolic protein, a secreted protein, a nuclear protein, and a chaperon protein.

10. The viral vector of claim 6, wherein the vector is a tobacco mosaic viral vector.

11. The viral vector of claim 6 that is designated BSG1057 deposited with American Type Culture Collection accession number 20398.

12. A plant cell transformed with the viral vector of claim 6.

13. An isolated nucleic acid sequence of SEQ ID NO: 1.

14. An isolated nucleic acid sequence of SEQ ID NO: 2.

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